

I Claim:

Subt 3
1. A method for preserving a cell or tissue specimen comprising the steps of contacting the specimen with a solution comprising a non-permeating co-solute (characterized by) its ability to limit the amount of a permeating cryoprotectant to permeate into the specimen. ①

2. The method for preserving a cell or tissue specimen as claimed in claim 1, wherein the solution further comprises a permeating cryoprotectant and a non-permeating cryoprotectant. ②

3. The method for preserving a cell or tissue specimen as claimed in claim 1, further comprising the step of contacting the specimen with a cryopreservation solution comprising a permeating cryoprotectant, a non-permeating cryoprotectant and a non-permeating co-solute. ③

4. The method for preserving a cell or tissue specimen as claimed in claim 2, wherein the cryoprotectant is selected from the group consisting of dimethylsulfoxide, ethylene glycol, propylene glycol and glycerol. ④

5. The method for preserving a cell or tissue specimen as claimed in claim 2, wherein the non-permeating cryoprotectant is selected from the group consisting of dextrans, starches, polyethylene glycol, polyvinylpyrrolidone, Ficoll and peptides. ⑤

6. The method for preserving a cell or tissue specimen as claimed in claim 1, wherein the non-permeating co-solute is selected from the group consisting of an amino acid and derivatives thereof, a betaine, a carbohydrate and a sugar alcohol, wherein the carbohydrate is selected from the group consisting of an aldose monosaccharide, a ketose monosaccharide, an amino sugar, an alditol, an inositol, ⑥

aidonic, uronic and aldaric acids, disaccharides and polysaccharides.

5 7. The method for preserving a cell or tissue specimen as claimed in claim 1, wherein the total concentration of non-permeating co-solute in the co-solute solution is between 0.1 and 0.7 mol/l and is equal to a maximum possible concentration that does not substantially damage cells.

8. The method for preserving a cell or tissue specimen as claimed in claim 6, wherein the co-solute is an amino acid.

Subt 5
5 9. The method for preserving a cell or tissue specimen as claimed in claim 2, wherein the method is performed in two or more stages of contacting the sample with increasingly higher concentrations of the permeating cryoprotectant and the co-solute.

5 10. The method for preserving a cell or tissue specimen as claimed in claim 2, wherein the method is performed by simultaneously increasing concentrations of both the permeating cryoprotectant and the co-solute from an initial concentration to a final concentration according to a desired profile.

11. The method for preserving a cell or tissue specimen as claimed in claim 2, wherein the rehydration solution further comprises a permeating rehydration cryoprotectant.

Subt 6
5 12. The method for preserving a cell or tissue specimen as claimed in claim 11, further comprising the step of rehydrating the specimen by contacting the preserved specimen with a rehydration solution comprising a non-permeating rehydration co-solute (characterized by) its

ability to limit the amount of a permeating cryoprotectant to permeate into the specimen, such that cryoprotectant within the specimen is removed from cells of the specimen.

5 13. The method for preserving a cell or tissue specimen as claimed in claim 12, wherein the permeating rehydration cryoprotectant is selected from the group consisting of dimethylsulfoxide, ethylene glycol, propylene glycol and glycerol.

5 14. The method for preserving a cell or tissue specimen as claimed in claim 12, wherein the rehydration step is performed by simultaneously decreasing concentrations of both the permeating rehydration cryoprotectant and the ^{non-permeating} rehydration co-solute from an initial concentration to a final concentration according to a desired profile.

5 15. The method for preserving a cell or tissue specimen as claimed in claim 12, wherein the non-permeating rehydration co-solute is selected from the group consisting of an amino acid (and) derivatives thereof, a betaine, a carbohydrate and a sugar alcohol, wherein the carbohydrate is selected from the group consisting of an aldose monosaccharide, a ketose monosaccharide, an amino sugar, an alditol, an inositol, aldonic, uronic and aldonic acids, disaccharides and polysaccharides.

16. The method for preserving a cell or tissue specimen as claimed in claim 1, wherein the contacting step is performed at room temperature or higher.

17. The method for preserving a cell or tissue sample as claimed in claim 1, wherein the specimen can be stably stored at a temperature greater than 4°C.

18. A cryopreservation solution for use in cryopreserving a cell or tissue specimen comprising a permeating cryoprotectant, a non-permeating cryoprotectant and a non-permeating co-solute.

19. The cryopreservation solution as claimed in claim 18, wherein the permeating cryoprotectant is selected from the group consisting of dimethylsulfoxide, ethylene glycol, propylene glycol and glycerol.

20. The cryopreservation solution as claimed in claim 18, wherein the non-permeating cryoprotectant is selected from the group consisting of dextrans, starches, polyethylene glycol, polyvinylpyrrolidone, Ficoll and peptides.

21. The cryopreservation solution as claimed in claim 18, wherein the non-permeating co-solute is selected from the group consisting of an amino acid and derivatives thereof a betaine, a carbohydrate and a sugar alcohol, wherein the carbohydrate is selected from the group consisting of an aldose monosaccharide, a ketose monosaccharide, an amino sugar, an alditol, an inositol, aldonic, uronic and aldaric acids, disaccharides and polysaccharides.

22. A rehydration solution for use in rehydrating cryopreserved cell or tissue specimen comprising a permeating rehydration cryoprotectant and a non-permeating rehydration co-solute.

23. The rehydration solution as claimed in claim 22, wherein the permeating rehydration cryoprotectant is selected from the group consisting of dimethylsulfoxide, ethylene glycol, propylene glycol and glycerol.

24. The rehydration solution as claimed in claim 22, wherein the non-permeating rehydration co-solute is selected from the group consisting of an amino acid and derivatives thereof, a betaine, a carbohydrate and a sugar alcohol, wherein the carbohydrate is selected from the group consisting of an aldose monosaccharide, a ketose monosaccharide, an amino sugar, an alditol, an inositol, aidonic, uronic and aldaric acids, disaccharides and polysaccharides.

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